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(54) GROWTH HORMONE CRYSTALS AND A PROCESS FOR PRODUCTION OF THESE GH-CRYSTALS.

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Description

The present invention concerns a method of producing growth hormone crystals in the presence of cations, novel growth hormone crystals and pharmaceutical preparations containing such novel crystals.

Background of the invention

The growth hormones (GH) from man and from the common domestic animals are proteins of approximately 191 amino acids, synthesized and secreted from the anterior lope of the pituitary. The growth hormone is a key hormone involved in the regulation of not only somatic growth, but also in the regulation of metabolism of proteins, carbohydrates and lipids.

During the past 40 years or more much attention has been devoted to the unravelling of the biochemical function of the growth hormones from various species. The reason for this interest in the molecular function of this protein rests upon the commercial interests from both veterinarian and medical circles. The GH gene has now been cloned and human growth hormone (hGH) and Met-hGH are currently beeing produced biosynthetically by the use of both bacteria and mammalian cell cultures.

Pharmaceutical preparations of GH tend to be unstable. Degradation products such as deamidated or sulfoxydated products and dimer or polymer forms are generated - especially in solutions of GH. Therefore, today GH is lyophilized and stored in the lyophilized form at 4°C until it is reconstituted by the patient, before start of use.

The reconstituted preparations are preferably stored at 4°C to minimize degradation in solution. However some degradation will take place during such storage which can be for a period of up to about 14 days. There is thus a need in the art for more stable preparations of GH.

It would also be an advantage to avoid the lyophilization step in the production of GH preparations. Lyophilization is a time consuming and costly process and also often a limiting procedure due to the capacity of the freeze drier.

The present invention is based on the surprising recognition that the above needs are fulfilled by means of a crystallization step in the production of GH.

Although readily available in quantities sufficient for crystallization, GH has so far eluded succesfull crystallization. Micro crystals, or amorphous material have been reported from a variety of sources: (Jones et al., Bio-Technology (1987) 5, 499 - 500; Wilhelmi et al., J.Biol.Chem. (1984) 176, 735 - 745; Clarkson et al., J.Mol.Biol. (1989) 208, 719 - 721; Bell et al., J.Biol.Chem. (1985) 260, 8520 - 8525 and V.L. Spitsberg, Analyticol Biochemistry, 1987, 160, 489 - 495)

The hanging drop method is the most common method in use for this purpose. Apparently due to heterogenicity among growth hormone preparations the size and the shape of the crystals have been reported to vary significantly. The largest crystals have been reported by Jones et al. (1987). For their successfull experiments they used a mixture of polyethylene glycol 3500 and beta octyl glucoside at neutral pH. Clarkson et al. (1989) reported that the use of lower alcohols and acetone permitted the generation of crystals of 0.001 to 0.005 cubic mm with varying shapes. None of the known methods are however suitable for commercial production of GH crystals a.o. due to the fact that growth times of from several weeks up to one year are needed.

Bovine growth hormone has been formulated for veterinarian use in a mixture of divalent ions and an oil (EP 343 696). By addition of $ZnCl_2$ to either bovine or ovine growth hormone in the presence of lipids undefined particles were produced to form a prolonged release formulation. The growth hormone was dispersed in the carrier in such a way as to trap 1 to 4 Zn molecules per growth hormone molecule. The solutions were prepared in the presence of varying concentrations of denaturing solutes (1 to 4 M of urea) at high pH (9.5). A reproduction of this process with hGH has shown that it is not possible to produce crystals in this way.

From the literature it is well known that the presence of divalent cations during the process of crystallization permits not only excellent orientation during analysis, but also improved physical conditions for the crystallization of insulin (e.g. US pat. no. 2174862). Growth hormone is, however, more than three times larger than insulin and has a totally different conformation. Surprisingly the addition of cations to solutions containing hGH have now permitted the generation of stable, uniform crystals of the growth hormone in high yields. Furthermore, the time used for the formation of high quality hGH crystals is relatively short.

Summary of the invention

In its broadest aspect the present invention is related to a process for production of cation crystals of GH or GH derivatives, comprising the following steps:

a) to a solution of GH or derivatives thereof is added cations of inorganic or organic nature and an organic solvent or a mixture of organic solvents at a pH between 5.0 and 6.8,

b) growing of crystals at a temperature from about 0 to about 30 ° C, and

c) isolation of the cation crystals by known means.

In the present context GH is intended to cover all species of GH including human, bovine, porcine, ovine, salmon, trout or tuna. GH derivatives are intended to cover GH of human or animal species with minor variation in the protein sequence. Thus a few amino acid residues may have been deleted or replaced by other amino acid residues. Also covered is truncated forms of growth hormone and derivatives thereof as well as growth hormones with amino acid residues added to the N- and/or C-terminal end of the protein, such as Met-hGH.

The process according to the present invention has for the first time made it possible to produce chemically stable and uniform cation-GH crystals. Also, the present process enables production of both larger and smaller crystals of growth hormone, as the need may be.

The pH in step a) is preferably 5.8 to 6.5, and most preferably from 6.0 to 6.5.

According to a preferred embodiment of the present invention the growth hormone is of human nature.

The cations may be of inorganic or organic nature. Divalent cations are preferred and of these an inorganic cation such as Zn^{++} has turned out to be well suited for the fast formation of stable GH crystals. Also mixtures of these cations can be used.

The cation should be added in an amount providing fast and efficient formation of well defined crystals. The upper limit for the amount of added cation is the amount which would cause unspecific precipitation of substantial amounts of amorpheous material.

If Zn^{++} is used, suitable concentrations will typically be from about 0.2 to 10 mol Zn^{++}/mol GH. However, if the crystallization reaction mixture contains a buffer or other compound which is capable of binding some of the cation, e.g. in a complexed form, greater concentration of the cation will be needed because some of the cation will not be available for the crystallization process.

Zn^{++} will preferably be used in an amount which will cause formation of GH crystals with a molar ratio between Zn^{++} and GH from about 0.2 to about 10, preferably from about 0.5 to about 5 and more preferably from about 0.5 to about 2.

In a preferred embodiment of the invention the organic solvent added in step a) may be chosen from the group consisting of short chained aliphatic, cyclic or aromatic alcohols and ketones. Suitable organic solvents are acetone, methanol, ethanol and 2-propanol. A preferred organic solvent is ethanol or acetone. The concentration of the organic solvent may be from 0.1 to 50% v/v, preferably from 0.1 to 30%, more preferably from 0.1 to 20%, even more preferably from 5 to 15% and most preferred from 6 to 12% v/v.

The present process may be used as a fast and efficient down stream processing of the growth hormone in question, due to the formation of crystals in large volumes of solutions.

The present invention is also related to novel cationic crystals of GH or GH derivatives.

The crystals are preferably hGH crystals or crystals of derivatives of hGH. The cation is preferably Zn^{++} and the molar ration between Zn^{++} and GH will typically be from about 0.2 to 10, preferably from 0.5 to 5 and more preferably from 0.5 to 2.0. The size of the crystals will be dependent on the Zn^{++} to GH ratio and the choice and content of solvent used in the process.

hGH crystals according to the present invention have been shown to have a biological potency similar to that of a solubilized hGH standard in in vitro and in vivo tests. The novel GH crystals can thus be used for the same indications as the commercially available hGH preparation.

Pharmaceutical preparations containing the novel GH crystals will typically be solutions or suspensions and may contain the usual adjuvants and additives used for pharmaceutical hGH preparations, such as buffers, glycerol and preservatives. The preparations may be administered in the same way as the commercial hGH preparations. The crystals may also be formulated as dried crystals which will then have to be reconstituted before start of use.

The pharmaceutical preparations containing the novel GH crystals have surprisingly a very high chemical stability compared with preparations made from commercially available GH.

The present invention therefore provides for a possibility of production of pharmaceutical preparations that are more convenient, especially for the patients. Due to the high stability of the crystals in suspension, the present invention will as an example make it possible to produce ready to use pharmaceutical preparations in the form of suspensions which will not need to be reconstituted by the patients before use.

In a further aspect the invention provides a valuable tool for production and purification purposes of GH.

Detailed description of the invention

The starting material, the growth hormone that may be of any origin and if desired derivatized in solution, is adjusted to a concentration preferably greater than about 0.1 mg/ml, more preferably from about 4 to about 7 mg/ml and most preferred about 6 mg/ml. The pH will preferably be from 6.0 to 6.3.

To the above mentioned solution may be added an organic solvent. A preferred organic solvent is ethanol in a concentration which may vary between 0,1 and 20%, preferably 5 and 15%, and most preferred 6 and 12%.

Other solvents such as acetone, methanol or propanol may be used alone or as a mixture instead of or together with ethanol in a concentration within the range of from 1 to 50%.

Cations of inorganic or organic nature, or mixtures thereof are then added to the resulting solution.

A preferred cation is Zn^{++} which will normally be used in a concentration from 0.5 to 10 mol/mol GH, preferably from 1.0 to 3.0 mol/mol GH, more preferred from 1.1 to 2.2 mol/mol GH and most preferred from 1.2 to 2.0 mol/mol GH.

If cations of inorganic nature other than Zn^{++} are used, the concentration may be varied between 0.5 and 10 mol/mol GH.

The crystals are then grown for a period of from 1 to 120 hrs. preferably 5-72 hrs., most preferred 20-48 hrs., and at a temperature of between 0 and 30 °C, preferably from 4 to 25 °C.

The crystals may be recovered by centrifugation or filtration, followed by washing and/or freeze drying to remove remaining organic solvents.

Pharmaceutical preparations of dried crystals or crystals in suspension can now be formulated by using various selected buffers and other pharmaceutically acceptable additives.

The invention is further illustrated but not limited by the following examples:

Example 1

Crystallization of hGH in the presence of Zn^{++}.

500 ml of hGH solution produced according to H. Dalbøge et al., Bio-Technology (1987), 5, 161 - 164, in a concentration of 6 mg/ml was incubated in 10 mM phosphat buffer (NaH_2PO_4) and adjusted to pH 6.1 with H_3PO_4. Acetone was added to a final concentration of 10% (v/v) and thereafter zinc acetate solution was added to a final concentration of 0.08 mg $ZnAc_2$, $2H_2O$/ml ~ 1.34 mol Zn^{++}/mol hGH.

The resulting solution was left at 15 °C for 20 hours, whereby crystals were allowed to form.

After this the crystals were recovered and washed 3 times with crystallization buffer without acetone. The crystallization was checked by microscopy and the size of the crystals were measured to 8-12 μm. A photomicrograph is shown in Figure 1.

The crystal yield of hGH was determined by solubilization of the washed crystals in 7M urea followed by ion exchange HPLC analysis.

The yield was found to be more than 50%.

Example 2

Example 1 was repeated with the exception that Met-hGH was used instead of hGH. The crystals recovered by this process were identical in shape and size to those obtained with hGH. The yield was more than 50%.

Example 3

Example 1 was repeated with the exception that the addition of acetone was omitted.

The crystals of hGH resulting from this procedure were much smaller than the crystals resulting from Example 1, less than 2 μm.

Example 4

Example 1 was repeated under conditions where acetone was exchanged with ethanol and temperature during growing period was 20 °C instead of 15 °C. All other experimental conditions were identical to those

described in example 1. By varying the ethanol concentration the optimal concentration was found to be 7.5% (v/v). The yield was increased to >80% if the motherfluid following initial crystallization for 16 hrs was supplemented with further 4% (v/v) ethanol and the crystallization temperature was lowered from 20° to 10°C over a period of 16 hrs. The size of the crystals were between 3 to 6 µm with a shape similar to that described in example 1.

Example 5

Determination of the amount of Zn bound in hGH crystals

Example 1 was repeated with the exception that ethanol in a concentration of 7.5% (v/v) was added instead of acetone and that crystals were allowed to form for 16 hrs at 20°C, then the crystals were separated from the motherfluid by centrifugation and washed once with 10 mM phosphate buffer. The crystals were solubilized by raising the pH to 8.0 with NaOH. The hGH was measured by ion exchange HPLC or by UV determination. The Zn concentration was measured by atomic absorption and the results were compared with those values obtained for the total crystal suspension. The ratio of bound Zn to hGH was found to be 1.9 mole of Zn per mole of hGH.

Example 6

Formulation of a Pharmaceutical Preparation Containing hGH:

Crystals were grown as described in example 5 and stored at 4°C. The crystals were then isolated by centrifugation and subsequent removal of the motherfluid. Then the crystals were freeze dried over night to achieve dry crystals with no remaining organic solvent. A pharmaceutical suspension of the dried crystals was prepared according to the following formulation:

hGH crystals	1.3 mg/ml
$NaH_2PO_4,2H_2O$	3.0 mg/ml
$Zn(Ac)_2,H_2O$	0.1 mg/ml
Glycerol	15.0 mg/ml
Benzyl alcohol	15.0 mg/ml
pH was adjusted to 6.2.	

Example 7

Example 6 was repeated with the exception that $Zn(Ac)_2,H_2O$ was omitted, giving a suspension of the following formulation:

hGH crystals	1.3 mg/ml
$NaH_2PO_4,2H_2O$	3.0 mg/ml
Glycerol	15.0 mg/ml
Benzyl alcohol	15.0 mg/ml
pH was adjusted to 6.2.	

Example 8

The crystals were treated in the same way as in example 6 and the following suspension was formulated:

hGH crystals	1.3 mg/ml
$NaH_2PO_4,2H_2O$	2.5 mg/ml
NaCl	5.7 mg/ml
Benzyl alcohol	15.0 mg/ml
pH was adjusted to 6.2.	

Example 9

The crystals were treated in the same way as in example 6 and the following solution was prepared:

hGH crystals	1.3 mg/ml
$NaH_2PO_4,2H_2O$	2.14 mg/ml
NaCl	9.0 mg/ml
pH was adjusted to 6.1.	

Example 10

Tibia test

To estimate the in vivo biological potency of the hGH crystals prepared according to the invention a tibia test was performed using hypophysectomized rats. The test was performed in accordance with the method described in the European Pharmacopoeia.

Two preparations of hGH crystals produced according to example 1 and formulated as preparations according to exampel 9 (F-7 and F-8) each containing an estimated amount equivalent to 4 IU were tested against a dissolved standard hGH preparation.

The following results were obtained:

Table 1

The potency of the preparations F-7 and F-8			
Test preparat.	Potency % of std.	IU/vial	95% confid. limits, % of std.
F-7	90.1	3.9	87.6 - 114.1
F-8	103.8	4.5	90.6 - 110.4
Std. hGH 1986	≡ 100.0	≡ 4.4	-

From the performed test it can be concluded that the hGH crystals according to the invention are equally biological potent as the solubilized hGH standard and therefore will have a bioavailability equal to that of usual solubilized hGH.

Example 11

hGH crystals were grown as described in example 5. Immediately before use a suspension was prepared by centrifugation of the crystals, subsequent removal of the motherfluid, and resuspension of the crystals in sterile 10 mM NaH_2PO_4, pH 6.2 giving a final concentration of 0.16 mg hGH/ml suspension.

The suspension was used to estimate the potency of the hGH crystal preparation in a weight gain assay. The test was performed in accordance with the method described in the European Pharmacopoeia, with the exception that the time of dosing was prolonged to 10 days in order to optimize the biological response.

Two preparations of hGH crystals were used, each containing the same amount of hGH protein as the preparations of a growth hormone standard, which they were tested against. The standard was a

reconstituted freeze-dried hGH preparation. All the animals received the same amount of hGH.

The potency of the hGH crystal preparations were found to be 92.6% of the standard. The 95% confidence limits were 79.1 - 126.4% of the standard.

The hGH crystal preparation was thus shown to have a biological potency equal to that of the solubilized hGH standard.

Example 12

Stability of hGH crystals stored in suspension for 6 months at 22-24°C.

The crystals were formed as described in Example 1 with the exception that 7.5% (v/v) acetone was added instead of 10%.

The crystals were allowed to remain in suspension in the mother fluid for 6 months at 22-24°C. A sample of hGH crystals were removed by centrifugation, washed once with crystallization buffer without acetone and solubilized by raising the pH to 8.0.

The solublized hGH crystals were subjected to analysis on ion exchange HPLC and GPC for detection of desamido and split forms or dimers and polymers, respectively.

When the data were compared with those of a reconstituted lyophilized hGH preparation stored at 25°C for 32 days the content of the main peak of hGH in reconstituted hGH crystals was superior to reconstituted lyophilized hGH, stored under comparable conditions (see table 2).

Table 2

	Reconstituted hGH	Crystals
Storage	25°C 32 days	22-24°C 6 months
Main peak on IE-HPLC (%)	71.2	92.3
Dimer (%)	0.7	1.2
Polymer (%)	0.3	0.3
Desamido (%)	25.9	5.0
Didesamido (%)	2.9	1.8
Split form (%)	-	-

Claims

Claims for the following Contracting States : AT, BE, CH, DE, DK, FR, GB, GR, IT, LI, LU, NL, SE

1. A process for production of cation crystals of GH or of GH derivatives, comprising the following steps:
    a) to a solution of GH or derivatives thereof is added cations of inorganic or organic nature and an organic solvent or a mixture of organic solvents at a pH between 5.0 and 6.8,
    b) growing of crystals at a temperature from about 0 to about 30°C, and
    c) isolation of the cation crystals by known means.

2. A process according to claim 1, wherein the pH in step a) is from 5.8 to 6.5, preferably from 6.0 to 6.5.

3. A process according to claim 1, wherein the organic solvent is selected from the group consisting of short chained aliphatic, cyclic or aromatic alcohols or ketones.

4. A process according to claim 3, wherein the organic solvent is selected from the group consisting of acetone, methanol, ethanol and 2-propanol.

5. A process according to claim 4, wherein the organic solvent is ethanol or acetone.

6. A process according to any of claims 1 to 5 wherein the organic solvent is added in a concentration of about 0.1 to about 50% v/v.

7. A process according to claim 6, wherein the organic solvent is added in a concentration of 0.1 to 30%, preferably from 0.1 to 20%, more preferred from 5 to 15% and most preferred from 6 to 12%.

8. A process according to any of the preceding claims 1 to 7, wherein the cation is a divalent cation.

9. A process according to claim 8, wherein the divalent cation is Zn^{++}.

10. A process according to claim 9, wherein Zn^{++} is added in a concentration below the limit for unspecific precipitation of amorphous material.

11. A process according to claim 10, wherein Zn^{++} is added in a concentration from 0.5 to 10 mol Zn^{++}/mol GH.

12. A process according to claim 11 wherein the concentration of Zn^{++} is from 1.0 to 3.0 mol Zn^{++}/mol GH, more preferred from 1.1 to 2.2 mol Zn^{++}/mol GH and most preferred from 1.2 to 2.0 mol Zn^{++}/mol GH.

13. A process according to any of the preceding claims, wherein the growth hormone is hGH or derivatives thereof.

14. A process according to any of the preceding claims, wherein the temperature in step b) is from about 4 to about 25 °C.

15. Cation crystals of hGH or hGH derivatives.

16. Crystals according to claim 15, wherein the cation is Zn^{++}.

17. Crystals according to claim 16, wherein the molar ratio between Zn^{++} and GH is from about 0.2 to about 10, preferably from about 0.5 to 5 and more preferably from about 0.5 to 2.0.

18. Pharmaceutical preparations, characterized in that they contain crystals according to any of claims 15 to 17.

19. Use of a crystallization process according to claims 1 to 14 as a purification and/or isolation step in the manufacturing of GH.

Claims for the following Contracting State : ES

1. A process for production of cation crystals of GH or of GH derivatives, comprising the following steps:
   a) to a solution of GH or derivatives thereof is added cations of inorganic or organic nature and an organic solvent or a mixture of organic solvents at a pH between 5.0 and 6.8,
   b) growing of crystals at a temperature from about 0 to about 30 °C, and
   c) isolation of the cation crystals by known means.

2. A process according to claim 1, wherein the pH in step a) is from 5.8 to 6.5, preferably from 6.0 to 6.5.

3. A process according to claim 1, wherein the organic solvent is selected from the group consisting of short chained aliphatic, cyclic or aromatic alcohols or ketones.

4. A process according to claim 3, wherein the organic solvent is selected from the group consisting of acetone, methanol, ethanol and 2-propanol.

5. A process according to claim 4, wherein the organic solvent is ethanol or acetone.

6. A process according to any of claims 1 to 5 wherein the organic solvent is added in a concentration of about 0.1 to about 50% v/v.

7. A process according to claim 6, wherein the organic solvent is added in a concentration of 0.1 to 30%, preferably from 0.1 to 20%, more preferred from 5 to 15% and most preferred from 6 to 12%.

8. A process according to any of the preceding claims 1 to 7, wherein the cation is a divalent cation.

9. A process according to claim 8, wherein the divalent cation is Zn^{++}.

10. A process according to claim 9, wherein Zn^{++} is added in a concentration below the limit for unspecific precipitation of amorpheus material.

11. A process according to claim 10, wherein Zn^{++} is added in a concentration from 0.5 to 10 mol Zn^{++}/mol GH.

12. A process according to claim 11 wherein the concentration of Zn^{++} is from 1.0 to 3.0 mol Zn^{++}/mol GH, more preferred from 1.1 to 2.2 mol Zn^{++}/mol GH and most preferred from 1.2 to 2.0 mol Zn^{++}/mol GH.

13. A process according to any of the preceding claims, wherein the growth hormone is hGH or derivatives thereof.

14. A process according to any of the preceding claims, wherein the temperature in step b) is from about 4 to about 25 °C.

15. A process according to claim 11, wherein cation crystals of GH are produced wherein the molar ratio between Zn^{++} and GH is from about 0.2 to about 10, preferably from about 0.5 to 5 and more preferably from about 0.5 to 2.0.

Patentansprüche

Patentansprüche für folgende Vertragsstaaten : AT, BE, CH, DE, DK, FR, GB, GR, IT, LI, LU, NL, SE

1. Verfahren zur Herstellung von kationenhaltigen Kristallen aus Wachstumshormon (GH) oder aus Derivaten von GH, das folgende Schritte umfaßt:
   a) Zugabe anorganischer oder organischer Kationen und eines organischen Lösungsmittels bzw. eines Gemischs von organischen Lösungsmitteln zu einer Lösung von GH oder eines Derivates von GH bei einem pH-Wert von 5,0 bis 6,8,
   b) Züchtung von Kristallen bei einer Temperatur von etwa 0 bis etwa 30 °C,
   und
   c) Isolieren der kationenhaltigen Kristalle auf herkömmliche Weise.

2. Verfahren nach Anspruch 1, wobei der pH-Wert in Schritt a) 5,8 bis 6,5, vorzugsweise 6,0 bis 6,5, beträgt.

3. Verfahren nach Anspruch 1, wobei das organische Lösungsmittel unter kurzkettigen aliphatischen, cyclischen und aromatischen Alkoholen und Ketonen ausgewählt ist.

4. Verfahren nach Anspruch 3, wobei das organische Lösungsmittel unter Aceton, Methanol, Ethanol und 2-Propanol ausgewählt ist.

5. Verfahren nach Anspruch 4, wobei das organische Lösungsmittel Ethanol oder Aceton ist.

6. Verfahren nach einem der Ansprüche 1 bis 5, wobei das organische Lösungsmittel in einer Konzentration von etwa 0,1 bis etwa 50 Vol.-% zugegeben wird.

7. Verfahren nach Anspruch 6, wobei das organische Lösungsmittel in einer Konzentration von 0,1 bis 30 Vol.-%, vorzugsweise von 0,1 bis 20 Vol.-%, noch bevorzugter von 5 bis 15 Vol.-% und am bevorzugtesten von 6 bis 12 Vol.-% zugegeben wird.

8. Verfahren nach einem der vorhergehenden Ansprüche 1 bis 7, wobei das Kation ein zweiwertiges Kation ist.

9. Verfahren nach Anspruch 8, wobei das zweiwertige Kation Zn^{++} ist.

10. Verfahren nach Anspruch 9, wobei Zn^{++} in einer Konzentration, die unterhalb des Grenzwerts für die unspezifische Ausfällung von amorphem Material liegt, zugegeben wird.

11. Verfahren nach Anspruch 10, wobei Zn^{++} in einer Konzentration von 0,5 bis 10 mol Zn^{++} pro Mol GH zugegeben wird.

12. Verfahren nach Anspruch 11, wobei die Konzentration an Zn^{++} 1,0 bis 3,0 mol Zn^{++} pro Mol GH, vorzugsweise 1,1 bis 2,2 mol Zn^{++} pro Mol GH und am bevorzugtesten 1,2 bis 2,0 mol Zn^{++} pro Mol GH beträgt.

13. Verfahren nach einem der vorhergehenden Ansprüche, wobei das Wachstumshormon hGH oder ein Derivat davon ist.

14. Verfahren nach einem der vorhergehenden Ansprüche, wobei die Temperatur in Schritt b) etwa 4 bis etwa 25 °C beträgt.

15. Kationenhaltige Kristalle aus hGH oder aus Derivaten von hGH.

16. Kationenhaltige Kristalle nach Anspruch 15, wobei das Kation Zn^{++} ist.

17. Kationenhaltige Kristalle nach Anspruch 16, wobei das Molverhältnis von Zn^{++} zu GH etwa 0,2 bis etwa 10, vorzugsweise etwa 0,5 bis 5 und noch bevorzugter etwa 0,5 bis 2,0 beträgt.

18. Pharmazeutische Zusammensetzungen, dadurch gekennzeichnet, daß sie Kristalle nach einem der Ansprüche 15 bis 17 enthalten.

19. Anwendung des Kristallisationsverfahrens nach Anspruch 1 bis 14 als Reinigungs- und/oder Isolierungsschritt bei der Herstellung von GH.

Patentansprüche für folgenden Vertragsstaat : ES

1. Verfahren zur Herstellung von kationenhaltigen Kristallen aus Wachstumshormon (GH) oder aus Derivaten von GH, das folgende Schritte umfaßt:
a) Zugabe anorganischer oder organischer Kationen und eines organischen Lösungsmittels bzw. eines Gemischs von organischen Lösungsmitteln zu einer Lösung von GH oder eines Derivates von GH bei einem pH-Wert von 5,0 bis 6,8,,
b) Züchtung von Kristallen bei einer Temperatur von etwa 0 bis etwa 30 °C,
und
c) Isolieren der kationenhaltigen Kristalle auf herkömmliche Weise.

2. Verfahren nach Anspruch 1, wobei der pH-Wert in Schritt a) 5,8 bis 6,5, vorzugsweise 6,0 bis 6,5, beträgt.

3. Verfahren nach Anspruch 1, wobei das organische Lösungsmittel unter kurzkettigen aliphatischen, cyclischen und aromatischen Alkoholen und Ketonen ausgewählt ist.

4. Verfahren nach Anspruch 3, wobei das organische Lösungsmittel unter Aceton, Methanol, Ethanol und 2-Propanol ausgewählt ist.

5. Verfahren nach Anspruch 4, wobei das organische Lösungsmittel Ethanol oder Aceton ist.

6. Verfahren nach einem der Ansprüche 1 bis 5, wobei das organische Lösungsmittel in einer Konzentration von etwa 0,1 bis etwa 50 Vol.-% zugegeben wird.

7. Verfahren nach Anspruch 6, wobei das organische Lösungsmittel in einer Konzentration von 0,1 bis 30 Vol.-%, vorzugsweise von 0,1 bis 20 Vol.-%, noch bevorzugter von 5 bis 15 Vol.-% und am bevorzugtesten von 6 bis 12 Vol.-% zugegeben wird.

8. Verfahren nach einem der vorhergehenden Ansprüche 1 bis 7, wobei das Kation ein zweiwertiges Kation ist.

9. Verfahren nach Anspruch 8, wobei das zweiwertige Kation Zn^{++} ist.

10. Verfahren nach Anspruch 9, wobei Zn^{++} in einer Konzentration, die unterhalb des Grenzwerts für die unspezifische Ausfällung von amorphem Material liegt, zugegeben wird.

11. Verfahren nach Anspruch 10, wobei Zn^{++} in einer Konzentration von 0,5 bis 10 mol Zn^{++} pro Mol GH zugegeben wird.

12. Verfahren nach Anspruch 11, wobei die Konzentration an Zn^{++} 1,0 bis 3,0 mol Zn^{++} pro Mol GH, vorzugsweise 1,1 bis 2,2 mol Zn^{++} pro Mol GH und am bevorzugtesten 1,2 bis 2,0 mol Zn^{++} pro Mol GH beträgt.

13. Verfahren nach einem der vorhergehenden Ansprüche, wobei das Wachstumshormon hGH oder ein Derivat davon ist.

14. Verfahren nach einem der vorhergehenden Ansprüche, wobei die Temperatur in Schritt b) etwa 4 bis etwa 25 °C beträgt.

15. Verfahren nach Anspruch 11, wobei kationenhaltige GH-Kristalle hergestellt werden, in denen das Molverhältnis von Zn^{++} zu GH etwa 0,2 bis etwa 10, vorzugsweise etwa 0,5 bis 5 und bevorzugter etwa 0,5 bis 2,0 beträgt.

Revendications
Revendications pour les Etats contractants suivants : AT, BE, CH, DE, DK, FR, GB, GR, IT, LI, LU, NL, SE

1. Procédé pour la production de cristaux cation d'hormone de croissance (GH) ou de dérivés de l'hormone de croissance, comprenant les étapes suivantes :
a) à une solution de GH ou de ses dérivés, on ajoute des cations de nature organique ou minérale et un solvant organique ou un mélange de solvants organiques à un pH entre 5,0 et 6,8,
b) mise en culture des cristaux à une température allant d'environ 0 jusqu'à environ 30 °C, et
c) isolement des cristaux cation par des moyens connus.

2. Procédé selon la revendication 1, dans lequel le pH de l'étape a) va de 5,8 à 6,5, de préférence de 6,0 à 6,5.

3. Procédé selon la revendication 1, dans lequel le solvant organique est choisi dans le groupe constitué par des cétones ou alcools aliphatiques, cycliques ou aromatiques à chaîne courte.

4. Procédé selon la revendication 3, dans lequel le solvant organique est choisi dans le groupe constitué par l'acétone, le méthanol, l'éthanol et le 2-propanol.

5. Procédé selon la revendication 4, dans lequel le solvant organique est l'éthanol ou l'acétone.

6. Procédé selon l'une quelconque des revendications 1 à 5, dans lequel le solvant organique est ajouté dans une concentration d'environ 0,1 à environ 50 % v/v.

7. Procédé selon la revendication 6, dans lequel le solvant organique est ajouté dans une concentration de 0,1 à 30 %, de préférence de 0,1 à 20 %, de façon plus préférée de 5 à 15 % et de façon la plus préférée de 6 à 12 %.

8. Procédé selon l'une quelconque des revendications précédentes 1 à 7, dans lequel le cation est un cation divalent.

9. Procédé selon la revendication 8, dans lequel le cation divalent est Zn^{++}.

10. Procédé selon la revendication 9, dans lequel Zn^{++} est ajouté dans une concentration au-dessous de la limite pour la précipitation aspécifique de matériau amorphe.

11. Procédé selon la revendication 10, dans lequel Zn^{++} est ajouté dans une concentration de 0,5 à 10 moles Zn^{++}/mole GH.

12. Procédé selon la revendication 11, dans lequel la concentration de Zn^{++} va de 1,0 à 3,0 moles Zn^{++}/mole GH, de façon plus préférée de 1,1 jusqu'à 2,2 moles Zn^{++}/mole GH, et de façon la plus préférée de 1,2 jusqu'à 2,0 moles Zn^{++}/mole GH.

13. Procédé selon l'une quelconque des revendications précédentes, dans lequel l'hormone de croissance est l'hGH (hormone de croissance humaine) ou ses dérivés.

14. Procédé selon l'une quelconque des revendications précédentes, dans lequel la température de l'étape b) va d'environ 4 jusqu'à environ 25 ° C.

15. Cristaux cation de hGH (hormone de croissance humaine) ou des dérivés d'hGH.

16. Cristaux selon la revendication 15, dans lesquels le cation est Zn^{++}.

17. Cristaux selon la revendication 16, dans lesquels le rapport molaire entre Zn^{++} et GH va d'environ 0,2 jusqu'à environ 10, de préférence d'environ 0,5 jusqu'à 5 et de façon plus préférée d'environ 0,5 jusqu'à 2.

18. Préparations pharmaceutiques, caractérisées en ce qu'elles contiennent des cristaux selon l'une quelconque des revendications 15 à 17.

19. Utilisation d'un procédé de cristallisation selon les revendications 1 à 14 en tant qu'étape de purification et ou d'isolement dans la fabrication de GH.

Revendications pour l'Etat contractant suivant : ES

1. Procédé pour la production de cristaux cation d'hormone de croissance (GH) ou de dérivés de l'hormone de croissance, comprenant les étapes suivantes :
a) à une solution de GH ou de ses dérivés, on ajoute des cations de nature organique ou minérale et un solvant organique ou un mélange de solvants organiques à un pH entre 5,0 et 6,8,
b) mise en culture des cristaux à une température allant d'environ 0 jusqu'à environ 30 ° C, et
c) isolement des cristaux cation par des moyens connus.

2. Procédé selon la revendication 1, dans lequel le pH de l'étape a) va de 5,8 à 6,5, de préférence de 6,0 à 6,5.

3. Procédé selon la revendication 1, dans lequel le solvant organique est choisi dans le groupe constitué par des cétones ou alcools aliphatiques, cycliques ou aromatiques à chaîne courte.

4. Procédé selon la revendication 3, dans lequel le solvant organique est choisi dans le groupe constitué par l'acétone, le méthanol, l'éthanol et le 2-propanol.

5. Procédé selon la revendication 4, dans lequel le solvant organique est l'éthanol ou l'acétone.

6. Procédé selon l'une quelconque des revendications 1 à 5, dans lequel le solvant organique est ajouté dans une concentration d'environ 0,1 jusqu'à environ 50 % v/v.

7. Procédé selon la revendication 6, dans lequel le solvant organique est ajouté dans une concentration de 0,1 à 30 %, de préférence de 0,1 à 20 %, de façon plus préférée de 5 à 15 % et de façon la plus préférée de 6 à 12 %.

8. Procédé selon l'une quelconque des revendications précédentes 1 à 7, dans lequel le cation est un cation divalent.

9. Procédé selon la revendication 8, dans lequel le cation divalent est Zn^{++}.

10. Procédé selon la revendication 9, dans lequel Zn^{++} est ajouté dans une concentration au-dessous de la limite pour la précipitation aspécifique de matériau amorphe.

11. Procédé selon la revendication 10, dans lequel Zn^{++} est ajouté dans une concentration de 0,5 à 10 moles Zn^{++}/mole GH.

12. Procédé selon la revendication 11, dans lequel la concentration de Zn^{++} va de 1,0 à 3,0 moles Zn^{++}/mole GH, de façon plus préférée de 1,1 à 2,2 moles Zn^{++}/mole GH, et de façon la plus préférée de 1,2 à 2,0 moles Zn^{++}/mole GH.

13. Procédé selon l'une quelconque des revendications précédentes, dans lequel l'hormone de croissance est l'hGH (hormone de croissance humaine) ou ses dérivés.

14. Procédé selon l'une quelconque des revendications précédentes, dans lequel la température à l'étape b) va d'environ 4 jusqu'à environ 25 °C.

15. Procédé selon la revendication 11, dans lequel on produit des cristaux cation de GH (hormone de croissance), le rapport molaire entre Zn^{++} et GH se situant entre environ 0,2 jusqu'à environ 10, de préférence d'environ 0,5 à 5 et de façon plus préférée d'environ 0,5 à 2,0.

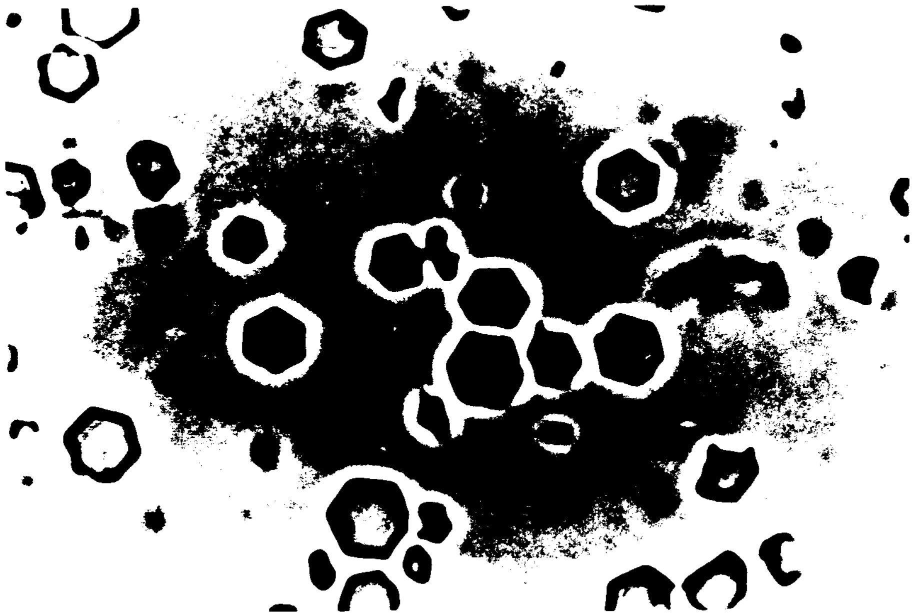

Fig. 1